REMARKS

Entry of the foregoing, reexamination and reconsideration of the above-identified application is respectfully requested.

Applicants note with appreciation the courtesies extended to Professor Svanborg as well as applicants' representatives during the personal interview with Examiner Holleran and Supervisory Examiner Caputa on December 3, 2002. The rejections of record and ways of overcoming these were discussed.

The pending claims 1-5 and 11-23 have been deleted by this amendment in favor of new claims 24-51. These claims are believed to more clearly define applicants' invention and are in keeping with the proposed claim language discussed during the interview. These claims find support in the originally filed application as follows:

24. Page 3, lines 12-19, page 3 line 21-25, page 9 lines 13-14.
25. Page 3, line 11.
26. Page 3, lines 12-16.
27. Page 3, lines 12-14 and 16-19.
28. Page 3, line 27, original claim 2.
29. Page 3, line 28, original claim 3.
30. Page 3, lines 28-29, original claim 4.
31. Page 3, lines 31-34, original claim 5.
32. Page 3, line 36.
33. Page 3, line 36 - page 4 line 2.
34. Page 4, lines 2-3.
35. Page 4, line 2-4, original claim 9.
36. Page 4, lines 4-7, original claim 10.
37. Original claim 1, combined with page 3, lines 12-19 and 21-25.
38. Page 4, line 1.
39. Page 3, line 37.
40. Page 4, line 2.
41. Original claim 8.
42. Page 4, line 2-4, original claim 9.
43. Page 4, lines 4-7, original claim 10.
44. Original claim 11.

45. Original claim 11.
46. Original claim 12.
47. Original claim 12.
48. Page 3, lines 12-19 and 21-25, original claim 17.
49. Original claim 18.
50. Original claim 19.
51. Original claim 19.
52. Original claim 18.

No new matter is thus added by the instant amendment.

Claims 1-5, 11-14, 17 and 20-23 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly not being enabled by the specification. This rejection is respectfully traversed.

The Official Action acknowledges that the specification enables methods of treating cancer wherein the claimed agent is directly applied to a tumor. However, it is asserted that methods of treating cancer where the agent is administered in any fashion to the patient are not enabled. This rejection is believed to be in error.

Applicants claims are now directed to a protein complex comprising an oligomeric form of α-lactalbumin (MAL) and either a cytotoxin or a labeling agent (claims 37-43), pharmaceutical compositions comprising same (claims 44-47), and methods of using such complexes (24-36 and 48-52). The complex and composition claims are fully enabled since the specification teaches how to make such complexes and compositions and how to use same. As acknowledged by the Official Action, at the very least, the specification enables one skilled in the art to use such complexes and compositions for direct application to the tumor. This in itself, is sufficient for enablement of the complex and composition claims.

The method of use claims are also fully enabled. As recited in claim 24, applicants claim a method for delivering a reagent into the nucleoplasm of a cell using a complex of MAL and the reagent. These method claims are in keeping with the recognized enablement of methods employing direct application of the complex to the tumor, as acknowledged in the Official Action. Claims 24-36 are thus fully enabled by the specification.

Claims 48-52 are also fully enabled by the specification. Claim 48 is directed to a method of treating cancer by administering an effective amount of the protein complex of claim 37, which comprises MAL and a cytotoxin. One skilled in the art could readily practice the claimed invention without undue experimentation. According to the Official Action, the cytotoxic agent may be toxic to the cell upon administration irrespective of the ability of MAL to target a cancer cell. However, it is believed that one skilled in the art would be able to practice the invention as claimed. One skilled in the art would be able to select an appropriate cytotoxic agent, or appropriate dose of an agent, which will not kill non-cancer cells to an unacceptable level upon administration. Selection of appropriate agents and dosages would be well within the ordinary skill of the art. Cytotoxic agents are well known in the art and their use has been extensively studied. No undue experimentation would thus be required for selecting an appropriate cytotoxic agent and dose thereof.

Similarly, claims 49-52 are enabled by the specification. Claims 49-52 are directed to diagnostic applications, where a protein complex together with a labeling agent is applied to a cell. In each of these claims, the complex is applied to the cell and the

presence of the labeling agent detected in the cell nucleus. Since these claims (1) do not encompass cytotoxic agents, and (2) observe/detect the complex in the cell nucleus, these claims are also fully enabled by the specification. These claims would not have the potential problem set forth in the Official Action of cytotoxic agents. These claims are thus fully enabled by the specification.

On page 5, last paragraph, it is mentioned that *in vivo* experiments are not provided. However, such experiments are not required. *See, In re Brana*, 34 USPQ2d 1436 (Fed. Cir. 1995) and the Patent Office's *Utility Guidelines*. Applicants provide in the specification *in vitro* data showing cell surface binding of MAL complexes (Example 2), nuclear uptake of MAL (Example 3), intracellular distribution of MAL (Example 4), effects of MAL complexes on isolated nuclei (Example 5), the role of the nuclear pore complex in the nuclear uptake of MAL (Example 6) and the role of Ca^{2+} for nuclear uptake of MAL and for induction of DNA fragmentation (Example 7). These examples all show the ability of MAL to be taken up by and penetrate cell nuclei.

As discussed in the specification, for example, at page 3, lines 10-20:

> It has now been found that MAL is taken up by susceptible cells (i.e. tumour cells) and accumulated in cell nuclei. This high uptake by the nucleus, combined with its oligomeric protein structure, means that MAL would provide a useful carrier for other moieties for example, cytotoxins or chemotherapeutic agents whose effect would supplement the effect of MAL in killing tumour cells, or diagnostic reagents such as dyes or radio- or other labels which would allow identification of tumour cells, whilst at the same time, allowing MAL to exert a killing effect on those cells.

MAL will thus be taken up by cells when administered.

As discussed at the interview, *in vivo* effects of MAL are being confirmed in a variety of ways. Some *in vivo* effects of MAL are shown in the paper "HAMLET Induces Apoptosis in Glioblastoma Cells and Delays Tumour Progression *In Vivo*," a draft of which was previously submitted by applicants. This paper shows that MAL is effective *in vivo*. Since MAL was shown to be able to target cells *in vivo*, one skilled in the art would recognize that, in accordance with the present invention, further reagents could be joined to MAL and the complex would similarly penetrate the cells and be effective *in vivo*.

In view of the above, the full scope of the claims now pending are believed to be enabled.

Withdrawal of the rejection of record is thus respectfully requested. Such action is believed to be in order.

Claims 1 and 11-13 have been rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Sabharwal et al as evidenced by Kuwajima. Claims 1 and 17 have also been rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Hakansson et al as evidenced by Kuwajima. These rejections are respectfully traversed.

Neither Sabharwal et al nor Hakansson et al teach or suggest the instantly claimed invention. Neither reference teaches a protein complex comprising MAL together with a further reagent, i.e., a labeling agent or a cytotoxic agent. As discussed by Professor Svanborg in her Declaration submitted herewith, neither of the references discloses or suggests such a complex as recited in the claims. The references do not disclose a MAL having cytotoxic activity or having a cytotoxic agent. As stated in the Declaration, at Paragraph 3:

Furthermore, as explained on page 10 of WO96/04920, we were able to produce multimeric α-lactalbumin with a similar biological activity (in that case anti-adhesive effects), from commercial human α-lactalbumin, by subjecting this to similar ion exchange chromatography, using a high (1M NaCl) salt gradient. This suggests that the biological activity is not a function of an uncharacterised cytotoxin present in milk.

Nor do the references disclose or suggest MAL with a labeling agent, as also recited in the instant claims.

More work was, therefore, done by the inventors to obtain the instantly claimed invention:

4. Following on from the work described in these references, we conducted more detailed structural analyses of the proteins described there, and came to the conclusion that the biologically active protein described in the Sabharwal et al. Hakansson et al. references listed in section (2) above is a folding variant of α-lactalbumin, which is stabilised by a co-factor such as oleic acid, which is present in casein.

In order to prove this hypothesis, we produced recombinant α-lactalbumin, by expression in *E. coli.* This protein was therefore free of any other components found in the casein-containing fraction of milk. We were then able to convert this pure protein to the biologically active form by first partially unfolding it using EDTA to remove calcium ions and to form the apo protein, and passing this down a column matrix which had been pretreated with oleic acid. This further work has been reported in Svennson et al, *PNAS* 97(8): 4221-4226 (2000), a copy of which is enclosed as Annex A.

The product of this process had the same biological and physical properties of the product obtained by Sabharwal et al. and Hakansson et al. and is, in my view, the same product. As reported in the paper, oleic acid and other lipid extracts at the concentrations found in this product was tested and found to have no cellular effects. Therefore it is clear that the oleic acid is not acting as a cytotoxin. Neither is oleic acid a label reagent.

5. It is clear to me that the product obtained and reported in the references does not include an uncharacterised cytotoxin. The biological effects observed was the result of the existence of the new folding variant of α-lactalbumin, which was induced in the references as a result of the fractionation and purification treatments to which the milk was subjected. In particular the ion exchange chromatography, combined

with elution at high salt concentration and the presence of the co-factor, resulted in the conversion of α-lactalbumin to the biologically active folding variant.

Thus, the references fail to disclose or even suggest a complex as instantly claimed. Nor are the claimed uses of the complex even suggested by the references. The rejections of record are based upon the hypothesis that the references "may" include an uncharacterized cytotoxic agent. The Declaration submitted herewith negates that possibility. Moreover, prior art rejections cannot be based upon mere possibility or probability. It must instead be shown that each element of the claimed invention is taught or suggested in the prior art.

A *prima facie* case of obviousness thus does not exist. Withdrawal of the rejection is thus respectfully requested and believed to be in order.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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